PATENT APPLICATION
ATTORNEY DOCKET NO. 27129/32407

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	)	For: METHOD FOR
	)	QUANTIFYING LBP IN BODY
White et al.	)	FLUIDS
	)	
Serial No: 08/377,391	)	Group Art Unit: 1806
	)	
Filed: January 24, 1995	)	Examiner: N. Johnson, Ph.D.

DECLARATION OF STEPHEN F. CARROLL, Ph.D., UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Stephen F. Carroll, Ph.D., hereby declare as follows that:

1. I received a B.A. in Biology from the University of California, Revelle College, San Diego, California in 1974 and a Ph.D. in Microbiology from the University of California, Los Angeles, California, in 1979. From 1980 to 1981 I was a post-doctoral fellow and from 1982 to 1984 I was an Assistant Research Microbiologist II and III in the Department of Microbiology at the University of California, Los Angeles. From 1984 to 1987, I was an Assistant Professor in the Department of Microbiology and Molecular Genetics at Harvard Medical School, Boston, Massachusetts. In 1987, I joined XOMA Corporation, Berkeley, California as a Director of Protein Chemistry in the Preclinical Science department. I became Director of Biological Chemistry in 1991, and Director of Preclinical Science in 1995. Since 1996, I have been the Vice President of Preclinical Research at XOMA Corporation. I am the author or co-author of more than 50 scientific publications and presentations, and the inventor or co-inventor on numerous U.S. and foreign issued patents, patent publications and applications.

2. I am a named co-inventor on the above-identified patent application. I have reviewed the art cited by the Examiner, namely, Ulevitch et al., U.S. Patent Nos. 5,245,013 (hereafter "the '013 patent") and 5,310,879 (hereafter "the '879 patent"), Schumann et al., *Science*, 249:1429-1431 (1990) (hereafter "Schumann"), Tobias et al., *J. Biol. Chem.*, 263:13479-13481 (1988) (hereafter "Tobias") and Geller et al., *Arch. Surg.*, 128:22-28 (1993) (hereafter "Geller"). I believe that I am qualified to discuss what those skilled in the art at the time the application was originally filed would understand from the disclosure of the above-identified application and from the art regarding lipopolysaccharide binding protein (LBP).

3. I make the following statements in paragraphs 4-5 to show that the LBP response in humans is specific to endotoxin exposure. I make the statements in paragraph 6 to further support the prognostic value of the LBP assay described in the application. I also make the statements in paragraph 7 to address the Examiner's stated concerns regarding assay of body fluids other than plasma or serum.

4. The data in the specification demonstrate that the LBP response in humans (*i.e.*, an elevation in LBP levels) is specifically triggered by exposure to endotoxin. When healthy adults were specifically challenged with 4 ng/kg of reference endotoxin, their plasma LBP levels began to rise about 6 hours after endotoxin (LPS) administration, peaked at about 10-12 hours, and returned to normal by one week post-LPS administration. See Example 8 and Figure 3. This was the first demonstration of an endotoxin-specific increase in LBP levels in humans.

5. The data in the specification further demonstrate that LBP levels directly correlate with exposure to biologically active endotoxin and that LBP elevation is a specific marker for endotoxin-associated conditions in humans. In contrast to acute phase proteins such as C-reactive protein and fibrinogen which are generally elevated in acute phase conditions, LBP levels are not generally elevated in acute phase conditions. Instead, LBP levels in humans are substantially elevated only in conditions associated with endotoxin exposure. Example 10 and Figures 5A-5C show that true acute phase proteins such as C-reactive protein and fibrinogen were elevated in both sepsis and rheumatoid arthritis, while LBP levels were significantly elevated only in subjects suffering from sepsis.

6. The value of the LBP assay as a prognostic indicator of outcome in conditions characterized by exposure to endotoxin, such as sepsis, is demonstrated by data in the specification. For example, Example 9 and Figure 4 of the specification show that among humans suffering from sepsis, patients with lower LBP levels (*e.g.*, lower than 46 μg/mL) had a significantly greater survival rate ($p=0.004$) than patients with higher LBP levels (*e.g.*, higher than 46 μg/mL). This has been confirmed by the report of Schumann et al., 36th Int'l Conf. on Antimicrobial Agents and Chemotherapy, New Orleans, LA, September 15-18, 1996 (hereafter "Schumann et al."), Exhibit 1 hereto, which states that:

> . . . *sustained high or increasing plasma LBP levels were significantly correlated with a fatal opucome [sic]* (Kendall's t:p <0.05). The data strongly support the view of LBP being an acute phase reactant that additionally may be a valuable parameter in monitoring course and severity of sepsis. [Emphasis added.]

Preliminary data on the LBP levels of 17 patients in ongoing clinical trials of the use of bactericidal/permeability-increasing protein (BPI) for treatment of severe meningococcemia in pediatric patients is consistent with the conclusion of Schumann et al.

7. The specification demonstrates LBP assays on diverse biological fluid samples, including human plasma, serum and synovial fluid samples. Additional experiments performed at XOMA Corporation confirmed that the immunoassay described in the specification could be used to measure LBP in other types of human body fluids, including urine, cerebrospinal fluid (CSF) and bronchoalveolar lavage (BAL) fluid samples. These experiments showed that the presence of added LBP is detectable in urine, CSF and BAL samples. In addition, endogenous LBP was measured in a CSF patient sample at 0.4 μg/mL and in one of three BAL patient samples at 0.016 μg/mL.

8. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: 2/11/97

Stephen F. Carroll, Ph.D.

LB14.

Resistance to Quinupristin/Dalfopristin Encountered During Treatment of Infections Caused by Vancomycin-Resistant *Enterococcus faecium*

CA WOOD[a], HD MANDLER[1], BE FRY-ARRIGHT[1], J SMITH-DAVIS[2] AI HARTSTEIN[2], and EA BLUMBERG[1]. Allegheny University of the Health Sciences, Philadelphia, PA[1] and Indiana University Medical Center, Indianapolis, IN[2]

Vancomycin-resistant *Enterococcus faecium* (VREF) is an increasingly common cause of nosocomial infection with few therapeutic alternatives. Over 18 months, we enrolled 24 patients (pts) in 2 open trials of quinupristin/dalfopristin (Q/D), an investigational streptogramin, for the treatment of VREF infections. Twenty-two pts were fully evaluable. Clinical and surveillance rectal VREF isolates were screened for susceptibility to Q/D by pristinamycin (P) disk diffusion testing (sponsor supplied surrogate for Q/D disks). Three pts (14%) had isolates recovered during treatment with reduced susceptibility (P zone <19 mm). All 3 pts were colonized, 1 was infected (#3, bacteremia). Isolates from these 3 pts were further characterized with Q/D MIC by microbroth dilution and pulsed-field gel electrophoresis (PFGE) of genomic DNA.

Pt. #: Infection	Culture source	Rx Day	P zone	Q/D MIC	PFGE pattern
1: Intra-abdominal	1 Abd abscess	6 Pre	28 mm	2.0 µg/mL	1, 2, and 3 indistinguishable, 4 different
	2 Rectal	11	29 mm	2.0 µg/mL	
	3 Rectal	21	17 mm	4.0 µg/mL	
	4 Rectal	88 Post	14 mm	4.0 µg/mL	
2: Neutropenia, bacteremia	1 Blood	1 Pre	26 mm	2.0 µg/mL	1 and 2 different
	2 Rectal	7	16 mm	8.0 µg/mL	
3: Neutropenia, bacteremia	1 Blood	2 Pre	23 mm	1.0 µg/mL	1, 2, and 3 indistinguishable
	2 Blood	10	14 mm	8.0 µg/mL	
	3 Rectal	16	12 mm	8.0 µg/mL	

The provisional resistance breakpoint for Q/D is ≥4.0 µg/mL. Our results illustrate emergence of resistance on therapy and either selection of preexisting resistant strains or emergence of resistance in unvexed strains. Resistance to Q/D must be anticipated.

LB15.

Emerging Vancomycin Resistance in Staphylococci Detected by MicroScan® Dried Overnight Panels.

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Dade MicroScan Inc., W. Sacramento, Cal.[1], Reston Hospital Center, Reston, Va.[2], Centers for Disease Control and Prevention, Atlanta, Ga.[3]

Two strains of gram-positive cocci were isolated from 2 positive blood cultures drawn from the same patient 5 days apart. The strains were tested in a MicroScan WalkAway® System on Dried Overnight Gram-Positive panels. Both strains were identified as *Staphylococcus epidermidis* and gave vancomycin (Va) MICs of 8 or 16, both of which are intermediate (I) interpretations. Repeat testing produced the same results. The hospital lab also tested Va by disk diffusion (16mm -I) [Va: S ≥ 12mm] and by AB BIODISK Etest® (8-I). Isolates were sent to Dade MicroScan (MS) for further evaluation. The MS reference lab tested the isolates on Dried Overnight panels, a frozen NCCLS reference panel and by disk diffusion (DD). The Va result on all the panels was 8 or 16 and the DD was 17mm - S. The identification (ID) was confirmed by conventional tube media. Because of the concern of emergence of resistance to Va in staphylococci, the isolates were sent to the CDC. The Va result (I) and the ID were confirmed by the CDC. The antibiogram of the two isolates differed only in that one strain was susceptible to clindamycin and erythromycin and the other strain was resistant to both. The 2 strains were resistant to penicillin, oxacillin, ciprofloxacin and trimethoprim/sulfamethoxazole and were susceptible to rifampin, tetracycline and chloramphenicol. This may be the first clinically significant blood culture isolate of *S. epidermidis* to demonstrate decreased susceptibility to vancomycin.

LB16.

Clindamycin Inhibits the Efficient Entry of Group A Streptococci (GAS) into HEp-2 Cells: Implications for GAS Disease Management Strategies

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During a recent outbreak of GAS severe invasive disease (SID) in our region, GAS isolates were obtained from asymptomatic carriers, and patients with pharyngitis and fatal SID. These GAS isolates were found to be identical by pulsed-field gel electrophoresis, suggesting a clonal source for the outbreak. The ability of these GAS isolates to enter HEp-2 cells was examined to determine how internalization correlated with these different disease states. A gentamicin/ampicillin *internalization* assay was performed in triplicate to measure the percent entry of GAS into HEp-2 cells. In 90 minutes, 4.7% (SEM ± 0.5) of the inoculum from a pharyngitis isolate, 8.3% (SEM ± 1.4) of the SID isolate and 15.5% (SEM ± 1.2%) of the carrier isolate were internalized compared to 0.6% (SEM ± 0.04%) of *L. monocytogenes* and 0.003% (SEM ± 0.0007%) of *E. coli* DH5α. Internalization was confirmed by electron microscopy. Further study of the carrier GAS isolates showed that internalization was reduced from 17% to 4% by exposure to clindamycin for 2 hours and to 1% by a 4 hour exposure. These data suggest that GAS possess internalization-associated factors, likely protein(s), and may explain why clindamycin, which inhibits protein synthesis, appears to be more effective than penicillin for eliminating GAS from the pharynx and improving outcomes in human cases and animal models of SID.

LB17.

Significantly Elevated Levels of Lipopolysaccharide Binding Protein (LBP) in Patients with Severe Sepsis: A Prospective Cohort Study with 109 surgical ICU Patients

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[1]Max-Delbruck-Centrum for Molecular Medicine (MDC) and Institute for Microbiology and Hygiene, University Hospital Charité, Humboldt-University, Berlin, and [2]University Hospital Benjamin Franklin, Free University of Berlin, Berlin, Germany

Host recognition of bacterial toxins and initiation of defense cascades have been shown to be crucial for pathogenesis of sepsis. LBP, a serum plasma protein recently identified (Schumann et al., Science 249, 1429) specifically binds bacterial LPS, transports it to the cellular CD14 receptor and thus enhances LPS-induced cellular effects, as the synthesis of pro-inflammatory cytokines. 63 patients with severe sepsis (classified by means of the APACHE III[TM] classification system) and 46 surgical ICU control patients were monitored daily for LBP levels, as well as for CRP, IL-6 and other parameters. LBP was determined by an enzyme-linked immunosorbent assay. Peak plasma levels of LBP in patients with a surgical trauma-induced inflammatory response were significantly elevated as compared to preoperative baseline controls (29.3 [3.43-58.6] mg/l vs. 7.81 [2.91-15.8] mg/l; student's t test: $p < 0.001$) but were significantly lower than LBP levels in patients with a septic inflammatory host response (83.0 [11.8-275] mg/l Mann-Whitney test: $p < 0.01$). Severity of infection also significantly correlated to LBP-levels ($p < 0.005$). No differences in peak plasma LBP concentrations were observed in patients with either gram-negative (n=6) or gram-positive blood cultures (n=7). Although there was no difference in peak LBP levels between survivors and non-survivors, sustained high or increasing plasma LBP levels were significantly correlated with a fatal outcome (Kendall's t: $p < 0.05$). The data strongly support the view of LBP being an acute phase reactant that additionally may be a valuable parameter to monitoring course and severity of sepsis.

LB18.

Role of Interleukin 8 in *B. henselae* Mediated Angiogenesis

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The direct stimulation of angiogenesis by localized bacterial infection is distinctly unusual and the mechanism of angiogenesis caused by *B.henselae* infection is little understood. There have been recent studies showing IL-8, a member of the CXC chemokine family, to play an important role in angiogenesis in various disease states such as rheumatoid arthritis and psoriasis. We investigated the the effects of viable and heat inactivated (HI) *B.henselae* on endothelial cell production of IL-8 in human umbilical vein endothelial cell cultures (HUVEC) and the effect of neutralizing antibody to IL-8 on proliferative response seen with viable *B.henselae*. Co-culturing HUVEC with viable *B.henselae* resulted in significant proliferation compared to HI and media controls, at 4 and 6 days post inoculation. Viable *B.henselae*, when compared to HI and media control, also caused significant production of IL-8 measured in tissue culture supernatants by ELISA. Northern blot analysis of IL-8 mRNA demonstrated similar results. Anti-IL-8 neutralizing antibody at concentrations of 25 and 12 ug per well resulted in significant reduction of the ability of viable *B.henselae* to stimulate endothelial cell proliferation when compared to bacteria alone ($p < 0.0001$). However, at 6 and 3 ug conc., the anti-IL-8 antibody failed to exhibit the antagonistic activity of *B.henselae* and results were similar to the use *B. bacteriae* alone. Incubation of *B.henselae* with an irrelevant antibody(anti-VPA, 50ug) at 24 ug concentration had no effect on its endothelial proliferative activity. Incubation of anti-IL-8 antibody with media control also had no effect on cell number when compared to media alone. In summary, viable but not heat inactivated *B.henselae* stimulated both endothelial cell proliferation and IL-8 production in vitro. The importance of *B.henselae* stimulation of endothelial IL-8 production in angiogenesis is further supported by the ability of anti-IL-8 antibody to inhibit endothelial cell proliferation.

LB19.

False-Positive Gen-Probe Direct *M. tuberculosis* Amplification Tests in Patients with Pulmonary *M. kansasii* Infection

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The Gen-Probe transcription-mediated amplification test (MTD) has recently been approved for use in the U.S. for the rapid diagnosis of pulmonary tuberculosis in patients with acid-fast smear-positive sputum. Three HIV-infected patients seen in our institution with abnormal chest radiographs and fluorochrome stain-positive sputum were evaluated for tuberculosis, including performance of the MTD test on expectorated sputum samples. Two of three patients' sputa were highly smear-positive (i.e., > 100 bacilli/high power field), while the third patient's sputum contained 6-10 bacilli/hpf. MTD results on the initial specimens from these patients ranged from 43,498 to 193,858 RLU. Gen-Probe defines values > 30,000 RLU as indicative of a positive test, i.e., the presence of *M. tuberculosis* RNA. All three patients' sputum cultures yielded growth of *M. kansasii* within 6-12 days. One patient's culture also contained *M. avium*, but none of the initial or follow-up cultures from the patients revealed *M. tuberculosis*. However, subsequent cultures from all three patients again revealed *M. kansasii*. Other sputum specimens from two of the patients that had only 1 + or 2 + smear positivity were MTD-negative in 2/3 instances. A fourth patient with 1 + smear positive sputum due to *M. kansasii* yielded a negative MTD test. Five cultures of *M. kansasii* (including these 4 patients' isolates and ATCC 12478), and cultures of several other species were examined at densities of 10^5-10^7 viable CFU/ml using the MTD test. All five isolates of *M. kansasii* and 3/3 isolates of *M. simiae* yielded false-positive tests with RLU values of 75,191 to 335,591. These results indicate that low-level false-positive MTD results can occur due to *M. kansasii* and possibly other Mycobacterium species in sputum. MTD RLU values of 30,000-400,000 should be interpreted with caution.

EXHIBIT 1

LB17.

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R.R. SCHUMANN*[1], J. ZWEIGNER[1,2], N. LAMPING[1], H.-J. GRAMM[2]

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